Previous studies on human T lymphocytes have shown that the degranulation marker CD107a can be used to define T cells, which show tumor specific cytolysis. To determine whether CD107a can be used in the murine setting as a marker for cytolysis, we first tested the degranulation activity on splenocytes from C57BL/6 (H-2b), FVB (H-2q) or Balb/c (H-2d) against Balb/c splenocytes. Results from the mixed lymphocyte cytolytic assays (MLCA) and cytolytic assays [^{51}Cr] showed a high degree of correlation with CD107a expression by FACS analyses. We next analyzed the degranulation activity of murine CIK cells by evaluating their capacity to mobilize CD107a to the cell membrane as a parameter of tumor specific cytolysis. CIK cells showed a high cytolytic capacity in ^{51}Cr assays when directed against syngeneic and allogeneic tumor cell lines (A20, P3X63, EL4, Yac-1 and P815). CIK cytotoxicity correlated with CD107a expression by FACS analyses. Immunofluorescence microscopy could confirm the existence of CD107a positive cytolytic granules in CIK cells from tumor cytotoxic assays. Using in vivo Bioluminescence Imaging (BLI) we characterized the migration pattern of CIK cells derived from a luciferase expressing transgenic mouse in tumor-bearing mice as compared to healthy controls. Furthermore, Balb/c mice bearing a sub-cutaneous A20 lymphoma, revealed tumor-specific homing of CIK cells and reduction of the tumor size within 3 days after i.v. injection. To identify tumor-specific CIK in vivo we are currently combining imaging techniques with CD107a degranulation analysis. These data will help to elucidate the complex cellular interaction mounting a graft-versus-tumor response, which depends on an effective combination of efficient trafficking, recognition and elimination of the tumor cells.

149

METHOTREXATE ALTERS HEMATOPOIETIC RECOVERY AND ENGRAFTMENT KINETICS WHEN ADDED TO CYCLOSPORINE FOR ACUTE GVHD PROPHYLAXIS AFTER REDUCED-INTENSITY STEM CELL TRANSPLANTATION

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Methotrexate (MTX) is a standard drug for graft-versus-host disease (GVHD) prophylaxis, but its effects on hematopoietic recovery and donor engraftment in the setting of reduced-intensity stem cell transplantation (RIST) are not well described. We compared these parameters and acute GVHD in patients with hematologic malignancies undergoing RIST on 2 consecutive clinical trials using identical reduced-intensity conditioning (fludarabine and cyclophosphamide). Group 1 included 50 patients receiving cyclosporine (CSA) alone for GVHD prophylaxis after RIST. Group 2 included 24 patients receiving CSA + MTX (5 mg/m^2 on days +1, +3, +6, +11) for GVHD prophylaxis. The groups were similar with respect to host immune status before RIST, age, and allograft dose of CD34$^+$ and CD3$^+$ cells. All patients in each group received salvage therapy with etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus fludarabine (EPOCH-F) prior to RIST. Fourteen patients in the CSA/MTX group also received rituximab with EPOCH-F. Hematopoietic recovery was delayed in the CSA/MTX group, with median neutrophil recovery (>500/μL) and platelet recovery (>100K/μL) occurring 3 and 6.5 days later, respectively, than in the CSA group. Donor engraftment was rapid and complete for most patients in each group (median total mononuclear cell chimerism 100% at day +28 for both groups), and no patient experienced graft rejection. However, full donor chimerism (>95%) was less common for CSA/MTX (75% at day +28, 71% at day +100) than for CSA (90% at day +28, 97% at day +100). Adding MTX to CSA decreased the incidence and severity of acute GVHD. Grade 2–4 acute GVHD occurred in 33/50 (66%) CSA patients, versus 9/24 (38%) CSA/MTX patients. Grade 3–4 GVHD affected 17/50 (34%) CSA patients, including 6 with grade 4 GVHD and 8 with steroid-refractory disease. In contrast, only 5/24 (21%) of CSA/MTX patients had grade 3 GVHD, while none had grade 4 or steroid-refractory GVHD. No treatment-related deaths occurred in the CSA/MTX group, but 14 CSA patients died of treatment-related complications. Thus, the addition of MTX to CSA markedly attenuates the GVH response post RIST, slowing donor engraftment and delaying hematopoietic recovery. These data illustrate the sensitivity of engraftment kinetics to the intensity of GVHD prophylaxis following RIST. Further studies should determine if CSA/MTX will be associated with an increased risk of disease progression after RIST.

150

GRADE 2–4 ACUTE GRAFT-VERSUS-HOST DISEASE AND EXTENSIVE CHRONIC GRAFT VERSUS HOST DISEASE ARE ASSOCIATED WITH SIGNIFICANTLY DECREASED SURVIVAL FOLLOWING REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

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Grade 2–4 acute graft-versus-host-disease (aGVHD) and extensive chronic GVHD (cGVHD) are associated with decreased overall survival (OS) following conventional allogeneic stem cell transplantation but their impact on reduced intensity transplantation remains controversial. We evaluated the impact of GVHD on survival in 112 high-risk patients, median age 50 years (range 18–70), with AML (n=29), MDS (n=19), CML (n=9), CLL (n=5), ALL (n=3), HD (n=10), NHL (n=16), MM (n=9), MMM (n=7), PNH (n=2), or renal cell carcinoma (n=3), who underwent a reduced intensity preparative regimen of extracorporeal photopheresis day −7, −6, pentostatin 8 mg/m^2 by continuous intravenous infusion day −5 through −4, and total body irradiation in three 200 cGy fractions day −3, −2, followed by allogeneic bone marrow stem cell infusion from 6/6 HLA matched related (n=70), 5/6 HLA matched related (n=10), or matched unrelated (n=32) donors. Thirty patients had prior autologous stem cell transplantation and 5 patients had prior conventional allogeneic stem cell transplantation. Full donor chimerism occurred in 89% of patients. Day 100 transplant related mortality (TRM) was 20%. The disease relapse rate was 22%. Grade 2, 3 or 4 aGVHD occurred in 7%, 6%, and 6% of patients respectively. The one-year OS by aGVHD grade was 70% for grade 0, 69% for grade 1, 29% for grade 2, 17% for grade 3, and 0% for grade 4. Grade 2–4 aGVHD was associated with higher day 100 TRM (37% vs 14%: p=0.03) and decreased median OS (5 months vs "not reached": p=0.001). Median OS was lower among patients with grade 2–4 aGVHD as compared to patients with grade 0–1 aGVHD in matched related donor transplants (5 months vs "not reached": p=0.002) and in mismatched related or matched unrelated donor transplants (6 months vs 35 months: p=0.0004). Patients with grade 2 or grade 3–4 aGVHD had similar median OS (6 months vs 3 months: p=0.24). Patients with limited or no cGVHD had similar one-year OS (90% vs 79%) but patients with extensive cGVHD had a significantly worse one-year OS (56% vs 82% p=0.0007). In conclusion, high-risk patients who undergo reduced intensity transplantation and develop grade 2–4 aGVHD or extensive cGVHD tolerate GVHD poorly, leading to significantly higher TRM and decreased overall survival. Patients with grade 2 aGVHD or grade 3–4 aGVHD have similarly poor OS. To improve survival, reduced intensity transplantation regimens that decrease the incidence of grade 2–4 aGVHD or extensive cGVHD need to be developed.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

151

SIGNIFICANCE OF ONE HUMAN LEUKOCYTE ANTIGEN MISMATCH ON OUTCOME OF NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION FROM RELATED DONORS USING THE MEXICAN SCHEDULE

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